and USP20 could function to reverse β2AR ubiquitination and thus, could be involved in the regulation of receptor trafficking. Indeed, coexpression of the β2AR with USP33 or USP20 (USP33/20) results in a dramatic reduction of isoproterenol-stimulated ubiquitination of the receptor. In contrast, similar coexpression of catalytically inactive USP33/20 mutants, which retains receptor binding, does not lead to any decrease in receptor ubiquitination. When HEK-293 cells expressing the β2AR are exposed to 10 μm isoproterenol for 6h, internalized β2ARs are found to colocalize with the lysosomal marker protein, LAMP2. Wild type USP33/20, but not the catalytically inactive mutants expression abolishes this colocalization. Moreover, in the presence of USP33/20, β2ARs are found to be redirected to the plasma membrane even in the continued presence of agonist thus inhibiting lysosomal trafficking while concomitantly promoting receptor recycling from the late endosomal compartments as well as resensitization of recycled receptors at the cell surface. Inhibition of both USP20 and USP33 expression by SiRNA prevents completely the recycling and resensitization of the receptor whereas inhibition of only one of the two enzymes does not. Finally, dissociation of constitutively bound USP20 and USP33 from the β2-AR immediately after agonist-stimulation and re-association upon prolonged agonist treatment allows receptors to first become ubiquitinated and then deubiquitinated thus providing a "trip switch" between degradative and recycling pathways at the late endosomal compartments. In conclusion, our results suggest that by de-ubiquitinating the internalized β2ARs, USP20 and USP33 prevent their lysosomal trafficking and promotes receptor recycling and resensitization to the plasma membrane. USP20 and USP33 thus serve as novel regulators that dictate both post-endocytic sorting as well as the intensity and extent of β2-AR signalling from the cell surface.ββ

J019

FUNCTIONAL CONSEQUENCES OF INACTIVATION OF L-TYPE CAV1.3 AND T-TYPE CAV3.1 CHANNELS ON IN VIVO PACEMAKER ACTIVITY AND CALCIUM CYCLING IN CARDIAC AUTOMATIC CELLS

A. TORRENTE [1], P. MESIRCA [1], A. FORT [1], P. NECO [3], A. GOMEZ [3], H. APTEL [1], J. STRIESSNIG [2], J. NARGEOT [1], M.-E. MANGONI [1]

[1] *IGF — Institut de Génomique Fonctionnelle, CNRS-UMR 5203 Inserm U661, University of Montpellier I & II, Montpellier, France*

[2] *Pharmacology and Toxicology Unit, Institute of Pharmacy and Centre for Molecular Biosciences Innsbruck, Innsbruck, Austria*

[3] *Inserm, U637, Physiopathologie Cardiovasculaire, Montpellier, France*

Cardiac automaticity, in normal conditions, is generated by the Sinoatrial Node (SAN) tissue.

In the last years, two pacemaker mechanisms are proposed: the "ion channels clock", based on the If current and the intracellular SR-dependent "Ca2+ clock", based on spontaneous diastolic Ca2+ release. In our opinion a relevant role is played also by the Cav1.3 (L-type Ca2+ current) and Cav3.1 (T-type Ca2+ current) channels. For this reason we studied the two mouse models Cav1.3 KO and Cav1.3/Cav3.1 double KO in vivo and in vitro. Electrocardiograms analysis showed a strong bradycardia ($p<0.01$). Respectively the Heart Rate (HR) of Cav1.3 KO and Cav1.3/Cav3.1 double KO are: 396.3 ± 39.4 bpm (N.4) and 360.9 ± 53.1 bpm (N.3); instead in WT the HR is 545.4 ± 29.6 bpm (N.9). In addition we detected in the two mouse model a marked arrhythmia determined by the presence of many blocks of first and second degree. Particularly Cav1.3/Cav3.1 double KO showed a dissociation of rhythm.

In vitro analysis was performed with the line scan technique on WT and Cav1.3 KO SAN cells to observe the dynamics of Ca2+ release. The results seem to indicate a difference in the frequency of transients that is reduced in Cav1.3 KO compared with WT. Moreover the transient length result to be longer in Cav1.3 KO 1248.9 ± 341.5 ms (N.8) than in WT 831.7 ± 259.7 ms (N.5). Interesting results are obtained also after the analysis of the differents parts of transient, as expected the recovery phase result longer in Cav1.3 KO 1053.5 ± 313.3 ms (N.5) than in WT 553.9 ± 178.5 ms (N.8), instead the ramp phase result longer in WT 192.5 ± 72.18 ms (N.8) than in Cav1.3 KO 83.25 ± 38 ms (N.5).

In conclusion our date indicate that the absence of Cav1.3 channel cause the slowdown of pace maker mechanism at the in vivo and in vitro level. More experiments are needed to draw clears conclusions about the role of Cav3.1 channel, but seem to be important in cardiac automaticity generation.

J020

A FUNCTIONAL ROLE FOR CAV1.3 CHANNELS IN MUSCARINIC REGULATION OF HEART RATE (HR) AND AUTOMATICITY IN PACEMAKER CELLS: EXPERIMENTAL RESULTS

P. MESIRCA [1], A. TORRENTE [1], L. MARGER [1], A. FORT [1], A. COHEN-SOLAL [1], A.-L. LEONI [2], J. STRIESSNIG [3], J. NARGEOT [1], M.-E. MANGONI [1]

[1] *IGF — Institut de Génomique Fonctionnelle, CNRS-UMR 5203 — Inserm U661, University of Montpellier I & II, Montpellier, France*

[2] *Institut du Thorax, Inserm U533, Nantes, France*

[3] *Abteilung Pharmakologie und Toxikologie, University of Innsbruck, Innsbruck, Austria*

Aim — To investigate the effects of different agonist for the muscarinic receptor on cardiac automaticity in Cav1.3-/-, Kir 3.4-/- and Cav1.3-/-Kir3.4-/- mice.

Method — In vivo: mice received methoxamine or CCPA once intraperitoneally and telemetric recordings were run continuously over 8 h. Quantitative ECG analyses were performed. In vitro: action potentials were recorded in isolated SAN cells before and after application of different doses of Ach, the variation in the spontaneous firing rate was evaluated.

Result — In vivo: in WT, in Cav1.3-/- and in Cav1.3-/-Kir3.4-/- mice, methoxamine (6 mg/kg) reduces the HR ($p<0.05$) of 35%, 53% and 48%, respectively, but it has not effect on Kir 3.4-/- mice. CCPA 0.01 mg/kg and 0.05 mg/kg have not effect on HR in all the mouse strains tested. CCPA 0.1 mg/kg reduces ($p<0.05$) the HR in WT, Cav1.3-/- and Cav1.3-/-Kir3.4-/- of 35%, 49% and 46% respectively, but not in Kir 3.4-/- mice.

In vitro: the pacemaker activity in WT SAN cells is strongly reduced with 10 nM Ach (42%, $p<0.01$) and it is stopped with 50 nM Ach, but it is not affected using 3 nM Ach. In Cav1.3-/-Kir3.4-/- cells, Ach has no effect ($p>0.05$) at each of the three doses tested. Concerning Kir3.4-/- cells, we have seen a reduction ($p<0.05$) of the firing rate both a 10 nM (18%) and at 50 nM (25%).

Conclusion — In vivo: Inactivation of Cav1.3 exacerbated the slowing of HR induced by agonists of the muscarinic signalling pathway in both Cav1.3-/- and Cav1.3-/-Kir3.4-/- compared to WT

counterparts. In contrast, inactivation of Kir3.4 channels reduced the responsiveness of HR to the same agonists. Thus, Kir3.4 channels are possibly the predominant mechanism controlling HR under vagal input. In vitro: The spontaneous firing rate is strongly reduced under Ach stimulation in SAN WT cells, but is not affected in Cav1.3-/-Kir3.4-/- SAN cells. Moreover, there is a small but significant reduction on firing rate in Kir3.4-/- cells. These data seems to indicate Cav1.3 channels as a potential contributor in the muscarinic regulation of automaticity in isolated SAN cells.

J021

REGULATION OF CARDIAC PROGENITOR CELLS DURING DEVELOPMENT

F. ROCHAIS [1], M. DANDONNEAU [1], R.-G. KELLY [1]

[1] *Institut de Biologie du Développement de Marseille Luminy — UMR 6216, Marseille, France*

Cardiac progenitor cells of the second heart field (SHF) contribute to the poles of the elongating embryonic heart. Failure or perturbation of SHF development leads to congenital heart defects. Recent studies have demonstrated the existence, in the postnatal heart, of resident cardiac progenitor cells that specifically express the transcription factor Islet1, a SHF marker, and that have the potential to differentiate into cardiomyocytes, smooth muscle and endothelial cells. Interestingly, several evidences suggest that these residual progenitor cells arise from the SHF.

Through analysis of a transgene integration site position effect we have identified the transcriptional repressor Hes1 as a novel regulator of SHF development. Hes1, a target gene of the Notch signaling pathway, is expressed SHF progenitor cells. Analysis of E15.5 Hes1-/- embryos reveals outflow tract alignment defects (ventricular septal defects and overriding aorta). At earlier developmental stages, Hes1-/- embryos display SHF proliferation defects, cardiac neural crest cells reduction and fail to completely extend the outflow tract. Thus these data reveal a role for Hes1, and potentially Notch signaling, in SHF development.

Given the importance of Isl1 as a marker of resident progenitor cells in the later heart we are analysing the role of known and novel regulators of the SHF (Hes1, Fgf10 and Tbx1) in the regulation of myocardial progenitor cell fate and in the definition of the critical niche occupied by residual cardiac progenitor cells in the forming and definitive heart.

In Fgf10-/- mice, outflow tract alignment occurs normally. However, Fgf10-/- hearts are highly dysmorphic. We thus hypothesize that Fgf10 deletion may affect the proliferative capacities of SHF progenitors in order to maintain the residual progenitor cells pool in the fetal heart. Initial results have revealed that whereas Fgf10-/- hearts undergo heart tube extension normally, proliferation is impaired.

Together, our results identify Hes1 as a novel regulator of SHF progenitor cell deployment and reveal a potential role of Fgf10 in regulating cardiac progenitor cell fate and cardiac growth during the fetal period. This study will increase our understanding of the molecular mechanisms governing the maintenance and differentiation of cardiac progenitor cells.

J022

EFFECTEURS DU RÉCEPTEUR NOTCH3 DANS LA CELLULE MUSCULAIRE LISSE DES PETITES ARTÈRES

C. FOUILLADE [1,2], M. MONET LEPRETRE [1,2], B. CARRETTE [1,2], V. DOMENGA [1,2], A. JOUTEL [1,2]

[1] *Inserm, U740, Paris, France*

[2] *Université Paris 7-Denis Diderot, Faculté de Médecine, Site Lariboisière, Paris, France*

Notch3 code pour un récepteur transmembranaire dont l'expression est fortement restreinte aux cellules musculaires lisses (CML) des petites artères. Des études génétiques chez l'homme et la souris ont démontré que Notch3 était un acteur clé dans la physiologie normale et la pathologie des petites artères. Chez l'homme, des mutations dominantes de Notch3 sont responsables de la maladie CADASIL, une forme héréditaire de maladie des petites artères cérébrales. Chez la souris, Notch3 est requis pour la maturation postnatale des petites artères, en contrôlant l'identité artérielle et le remodelage du cytosquelette des CML. Notch3 joue également un rôle clé dans la fonction normale des petites artères, en contrôlant les réponses myogéniques à la pression artérielle. L'activation de Notch3, dans la voie canonique, induit, par clivage protéolytique, la libération de son domaine intracellulaire qui se lie dans le noyau à RBP-Jk, favorisant la formation d'un complexe activateur de la transcription.

Notre objectif est d'identifier et de caractériser les effecteurs de Notch3 dans les petites artères.

Par une approche combinant transcriptome et Q-PCR sur des artères de souris Notch3KO et WT, nous avons identifié 11 gènes candidats. Leur niveau d'expression est significativement diminué dans les artères de souris Notch3KO, à un stade où il n'existe pas encore de lésions cellulaires visibles, et, cette diminution est corrigée par la réintroduction spécifiquement dans les CML d'une protéine Notch3WT mais pas par celle d'une protéine mutée défective pour la signalisation RBP-Jk. Les 6 gènes dont nous avons pu étudier le patron d'expression sont tous exprimés dans les CML artérielles. De façon remarquable, l'expression vasculaire est artérielle prédominante pour les 6 gènes et « petite artère » préférentielle pour 4 d'entre eux. De plus, chacun de ces gènes a un profil d'expression unique au niveau de l'arborisation artérielle et capillaire, mais, la superposition des différents profils recouvre celui de Notch3.

Les travaux en cours ont pour but de déterminer si les gènes candidats identifiés sont ou non des cibles primaires, RBPJk-dépendantes, et de caractériser histologiquement et fonctionnellement les mutants souris perte-de-fonction de ces gènes.

J023

ESTROGEN RECEPTOR-ALPHA MEDIATED THE ENDOTHELIAL NO RELEASE TRIGGERED BY DELPHINIDIN

M. CHALOPIN [1], A. TESSE [1], R. ANDRIANTSITOHAINA [1]

[1] *Inserm 771 — CNRS UMR 6214, Angers, France*

We previously reported that deletion of estrogen receptor-alpha (ER-alpha) abolishes endothelial response to wine polyphenols. The present study was designed to demonstrate that delphinidin, an anthocyanin that possess the same pharmacological profile than